Genitourin Med 1986; **62:** 88-92

Prevalence of *Chlamydia trachomatis* and other micro-organisms in women seeking abortions in Pittsburgh, Pennsylvania, United States of America

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SUMMARY The prevalence of *Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma hominis,* group B streptoccocus, herpes simplex virus, and *Neisseria gonorrhoeae* from cervical cultures obtained from 210 women seeking abortion in Pittsburgh, Pennsylvania, United States of America was 9·3%, 72·9%, 25·2%, 4·3%, 0·9%, and 0·9% respectively. Cultures from 40/203 (19·7%) patients failed to produce any of these organisms. *C trachomatis* isolation was not associated with age, race, marital status, average family income, number of sexual partners, history of gonorrhoea or syphilis, or previous pregnancies, live births, or abortions, and 82·4% of women with chlamydial infections had had no urogenital symptoms in the preceding six months. The highest concentration of *U urealyticaum* was 10^5 colour changing units (ccu)/ml, and about half of the positive ureaplasma cultures produced less than 10^3 ccu/ml of this organism.

Screening for *C trachomatis*, is encouraged to prevent neonatal morbidity and the common complication of pelvic inflammatory disease after abortion.

Introduction

Infections caused by *Chlamydia trachomatis* have acquired special importance in the past decade. This organism has been implicated in causing trachoma, adult and neonatal conjunctivitis, neonatal pneumonia and gastroentritis, non-gonococcal urethritis (NGU), post-gonococcal urethritis, cervicitis, pelvic inflammatory disease (PID), lymphogranuloma venereum, epididymitis, and proctitis, as well as other diseases.[1] The prevalence, incidence, and characteristics of chlamydial infections in women are still not fully understood, especially in pregnant women. Published reports have shown that infections with this bacterium are more prevalent in younger women. Little agreement exists among different investigators, however, as to other characteristics of chlamydial infected groups. In addition, published reports have focused attention mainly on patients attending clinics for the treatment of sexually transmitted diseases (STD), whereas other groups have not been studied as extensively. Geographical variations in the prevalence of this organism also still need to be defined. Furthermore, fewer studies have assessed the prevalence of other sexually transmitted organisms in women with chlamydial infection.

This study was designed to investigate the prevalence of *C trachomatis* in women attending a clinic for abortion on demand in Pittsburgh, Pennsylvania, United States of America, and the organism's association with *Ureaplasma urealyticum* and five other sexually transmissible micro-organisms. In addition, data on demographic and sexual history were examined in an attempt to identify more clearly patients at risk of chlamydial infection.

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Accepted for publication 27 September 1985

Patients, materials, and methods

POPULATION STUDIED

The study group consisted of women attending a clinic for abortion on demand (Women's Health Services; Pittsburgh, Pennsylvania, USA) during April and May 1981. After explaining the study and receiving signed informed consent, one of the clinic nurses obtained cervical specimens. Before the abortion procedure, one of the authors (CLG) obtained

demographic data and sexual histories from the patients using a standard questionnaire. Of the 243 women giving informed consent and from whom cervical cultures were obtained, 210 met the following study entrance criteria: (1) estimated age of gestation of 13 weeks or less as estimated from the last menstrual period; (2) completed questionnaire; and (3) no history of antibiotic treatment within the previous two weeks.

MICROBIOLOGICAL PROCEDURES

A culture for *Neisseria gonorrhoeae* and serum for syphilis serology (rapid plasma reagin (RPR) tests) were taken from each patient and handled by the clinic as part of their normal procedures. In addition, two cervical specimens were obtained from each patient. The first was taken using a calcium alginate swab and was placed into 1 ml chlamydial transport medium (2SP) as described previously by Smith *et al.*[2] No antibiotics were incorporated into the 2SP. This first swab was used for the isolation of group B streptococcus, *Mycoplasma hominis, U urealyticum,* and *C trachomatis.* The second specimen, taken to isolate herpes simplex virus (HSV), was obtained with a Dacron swab and placed into 1 ml viral transport medium, which consisted of Hank's balanced salt solution with 0·5% gelatin and 25 mmol/l N-2-hydroxyethylpiperazine-N[1]-2-ethanesulphonic acid (HEPES) buffer. Both cultures were kept on wet ice until transported to the laboratory within several hours.

On arrival in the laboratory, the first swab was expressed and used to inoculate several plates. Sheep blood agar and group B streptococcus pigment screening plates[3] were selected to identify this bacterium. The first plate was incubated aerobically, and the second anaerobically. Grouping of the streptococcal isolates was done by the Lancefield method.[4] To identify genital mycoplasmas, we used A7 agar and U9 broth as described previously in two reports by Shepard and Lunceford.[5,6] The amount of ureaplasma was measured by inoculating 0·2 ml of the 2SP medium into 1·8 ml of U9 broth and then performing five tenfold serial dilutions in U9 broth from this 10^{-1} concentration to 10^{-6}. All plates and broths were observed for seven days. The remaining material in the 2SP (about 0·5 ml, held at 4°C for up to 48 hours or frozen at -80°C) was used to inoculate McCoy cell monolayers to isolate chlamydiae.[7]

The viral transport medium tubes were either held at 4°C and processed within 48 hours or frozen at -80°C and processed for HSV within 72 hours. After the swab was discarded, 1 ml of an antibiotic solution (consisting of Hank's balanced salt solution with 400 units/ml penicillin G, 0·01 mg/ml streptomycin, 0·1 mg/ml gentamicin, and 0·01 mg/ml amphotericin B) was added to each tube, which was then refrigerated for 30 minutes. Then 0·2 ml of the mixture of viral transport medium and antibiotic solution was inoculated into each of two primary rabbit kidney, two human embryonic lung fibroblast, and one human foreskin fibroblast cell culture tubes (MA Bioproducts, Walkerville, Maryland, and HEM Research, Rockville, Maryland, USA). Tubes were observed daily for 10 to 14 days for the presence of typical cytopathic effect produced by HSV.

STATISTICAL ANALYSIS

Data were summarised and studied using a DB Master program (Stoneware Microcomputer Products; San Rafael, California USA) with an Apple II personal computer. The χ^2 test with Yates's correction was used for most statistical analyses.

Results

Eighty six percent of the patients were white, 75% were single, and the average duration of their education was 12·4 years. The mean age was 21·8 (range 14-39, median 21) years. The mean family income was $20 920 (range $2400 to $200 000, medium $15 000) for the 175 patients from whom this information was obtained.

Of the 210 women selected for the study, 105 (50%) were pregnant for the first time, 69 (32·9%) had had two or more previous pregnancies, and 68 (32·4%) had had previous induced abortions. During the preceding six months, 171 (81·4%) of the women had had only one sexual partner, 24 (11·4%) had had two, seven (3·3%) had had three, three (1·4%) had had four partners, and five (2·4%) had had five or more sexual partners.

Of the 210 cultures tested for chlamydiae, 27 (12·9%) proved unsatisfactory because of yeast contamination or toxicity to McCoy cells. Cultures from seven of these 27 patients were also negative for the other micro-organisms tested. Chlamydiae were found in 17 of the remaining 183 cultures, thus giving a prevalence rate of 9·3%. Of the 210 women, 72·9%

TABLE I *Prevalence of specific micro-organisms on serology or in cervical cultures from 210 women seeking abortions*

*Organism**	*No positive*	*No tested*	*Prevalence rate (%)*
Chlamydia trachomatis	17	183†	9·3
Ureaplasma urealyticum	153	210	72·9
Mycoplasma hominis	53	210	25·2
Group B streptococcus	9	210	4·3
Herpes simplex virus	2	210	0·9
Neisseria gonorrhoeae	2	210	0·9
Treponema pallidum	0	210	0
None of above	40	203†	19·7

* Data represent results of cultures for the first six organisms and serological tests for *Treponema pallidum.*
† Twenty seven specimens excluded because of contamination by yeast or toxicity to McCoy cell monolayers used in chlamydia test.

yielded *U urealyticum*, 25·2% *M hominis*, 4·3% group B streptococcus, 0·9% HSV, and 0·9% *N gonorrhoeae* (table I). Cultures from 40 (19·7%) of 203 women failed to yield any of the above organisms. Serological tests for syphilis were negative in all 210 patients.

At least two organisms were present in 68 women (32·4%), 51 of whom were positive for both of the genital mycoplasmas. Three organisms were isolated simultaneously from six women, all of whom yielded *U urealyticum*. *M hominis* was present in five of these six patients, and *C trachomatis* was found in four.

At least one other organism was isolated from 14 (82·4%) of 17 women from whom chlamydiae were isolated. Nine cultures grew *U urealyticum* and chlamydiae, one grew *M hominis* and chlamydiae, three grew both of the genital mycoplasmas and chlamydiae, and one yielded group B streptococcus, ureaplasmas, and chlamydiae. Of the 17 patients with chlamydial infection, 14 (82·4%) had had no urogenital symptoms in the preceding six months.

Eleven of the 17 (64%) women with chlamydiae and 63/166 (38%) non-infected patients were aged under 20 (χ^2 = 3·539; p<0·1). Differences between the chlamydia positive and negative patients in race, marital status, average family income, income group, or number of sexual partners were not significant.

No appreciable association was found between any of the organisms investigated and a history of vaginitis in the previous six months, urinary tract infections, vulvar symptoms, symptoms in the sexual partner, history of gonorrhoea or syphilis, or numbers of previous pregnancies, live births, or abortions.

Table II shows the concentrations of *U urealyticum* found in the 153 women with positive cultures for this organism. The highest concentration was 10^5 ccu/ml. About half the positive ureaplasma cultures produced less than 10^3 ccu/ml of this organism. Table III shows the factors associated with genital mycoplasma isolation. The presence of *U urealyticum* was significantly associated with marital status and also the number of sexual partners: married women had a lower prevalence rate for ureaplasma (48·0%) compared with unmarried women (76·2%) (p<0·01); and 15/15 (100%) patients with three or more sexual partners were positive for *U urealyticum* compared with 138/195 (70·8%) of women with fewer than three partners (p<0·05). Furthermore, in the 153 patients from whom ureaplasmas were isolated, cultures with 10^3 ccu/ml or more organisms were more likely to be from unmarried women than those with less than 10^3 ccu/ml. Women with *M hominis* were significantly older than those without the organism (p<0·05). The presence of *Mycoplasma hominis* was also associated with patients who had had more than one sexual partner in the preceding six months (p<0·05).

TABLE II *Concentrations of ureaplasma found in cervical cultures from 153 women seeking abortions*

Concentrations of ureaplasma (ccu/ml)	*No (%) with ureaplasma*	*Cumulative %*
10^1	27(17·6)	17·6
10^2	50(32·7)	50·3
10^3	50(32·7)	83·0
10^4	21(13·7)	96·7
10^5	5 (3·3)	100·0
Total	153 100·	100·0

ccu = Colour changing units.

TABLE III *Characteristics associated with isolation of genital mycolasmas*

Mycoplasmas isolated	*Characteristics studied*	*No (%) positive /No tested*	*X^2 Value*	*p Value*
Ureaplasma	Unmarried women	141/185 (76·2)	7·497	< 0·01
Ureaplasma	Married women	12/ 25 (48·0)		
Ureaplasma	⩾ 3 sexual partners in previous 6 months	15/ 15 (100)	4·818	< 0·05
Ureaplasma	1 or 2 sexual partners in previous 6 months	138/195 (70·7)		
⩾10^3 ccu/ml ureaplasma	Unmarried woman	71/128 (55·4)	9·154	< 0·01
⩾10^3 ccu/ml ureaplasma	Married women	5/ 25 (20·0)		
Mycoplasma hominis	⩾20 years old	39/129 (30·2)	4·266	< 0·05
Mycoplasma hominis	< 20 years old	14/ 81 (17·3)		
Mycoplasma hominis	2 sexual partners in previous 6 months	15/ 39 (38·5)	4·996	< 0·05
Mycoplasma hominis	1 sexual partner in previous 6 months	38/ 71 (22·2)		

ccu = Colour changing units.

Discussion

The prevalence of *C trachomatis* in non-pregnant women attending STD clinics ranges between 12% [8] and 33·3%,[9] whereas the organism has been isolated in 4·6% [10] to 22% [11] of women in gynaecological outpatient clinics. Hammerschlag *et al* reported that 6/322 (2%) of pregnant women in their study group were infected by chlamydiae, and that neonates born to four of these six women developed evidence of chlamydial infection.[12] Heggie *et al* found *C trachomatis* in 18% of patients attending a prenatal clinic in an urban hospital in Cleveland, Ohio, USA, and transmission to their neonates occurred in 27/95 (28%) of cases.[13] The prevalence of chlamydiae in pregnant women was 6·7% and 8·0% respectively in studies by Martin *et al* [14] and by Frommell *et al*.[15] Harrison *et al* reported a 6·9% prevalence rate in pregnant patients at the university obstetric service in Tucson, Arizona, USA.[16] More recently, the same authors found *C trachomatis* in 24-30% of obstetric patients from two groups of American Indians in New Mexico.[17] The chlamydial prevalence rate of 11% in pregnant patients in Atlanta, Georgia, USA, reported by Thompson *et al* was three times that of gonorrhoea and thirty times that of HSV in the same group.[18] In five separate reports, chlamydiae were isolated from 5·3%, 6·1%, 6·3%, 12·6%, and 13·8% of women seeking abortions in Scandinavian countries.[19-23] Our investigation is one of the first reports on the prevalence of chlamydiae in patients undergoing abortion in the USA. Thus the prevalence of *C trachomatis* in our population was similar to that in Scandanavian abortion groups and in the prenatal patients studied by Martin *et al* and Frommell *et al*. We found this micro-organism much less often, however, than was found in American Indians and the patients studied by Heggie *et al*.

Unlike other investigators,[15 19] we found no difference in age, race, or marital status between women yielding or not yielding chlamydiae. None of the other factors studied was associated with chlamydial infection. It therefore appears that the other demographic and sexual history information studied, either alone or combined, will not identify high risk patients. Neither did we observe any increased history of genital complaints in patients yielding two or three of the organisms that were sought.

From the prevalence of ureaplasmas in 72·9% of our patients, it appears that these organisms may be "normal flora" in the female genital tract in certain populations, as suggested by other studies.[19 24] In agreement with previous reports [25] we also found that *U urealyticum* was more common in unmarried and more promiscuous women. As stated earlier, the highest concentration found was 10^5 ccu/ml, and about half the ureaplasma positive cultures yielded less than 10^3 ccu/ml of the organism. In contrast, an unpublished observation from our clinical laboratory showed that positive cultures often produce 10^8 or 10^9 ccu/ml and only 7% produce less than 10^3 ccu/ml. Isolation and titrations were always performed using the same media in both laboratory facilities and following exactly the same protocol. This finding seems to indicate that patients with some kind of genital complaint have higher titres of *U urealyticum* than asymptomatic women. Though these populations are different, this suggests that more studies are needed to assess the role, if any, of the concentration of ureaplasmas in certain gynaecological syndromes.

Several new products are now being marketed for the rapid, simple, and less expensive diagnosis of chlamydial infections.[26 27] These infections are not only common in patients attending STD clinics and other groups, such as ours, but they are often asymptomatic, as we have observed.[11] Thus the means are now available to diagnose accurately and treat active infections as well as to identify asymptomatic and high risk patients. The feasibility of large scale screening of patients attending STD clinics, their sexual contacts, pregnant women, and other groups should be considered. It is common medical practice in the USA to screen routinely sexually active women for gonorrhoea at their first gynecological visit and also women uring the first prenatal examination. Screening for *C trachomatis* should probably now be considered to be as important as screening for gonorrhoea on these two occasions, as chlamydial infections are known to be three to 10 times more common than gonococcal infections. Chlamydial infections also often produce morbidity similar to that of gonorrhoea in both mothers and neonates. Confirmation and treatment of asymptomatic mothers infected with *C trachomatis*, other patients with symptomatic infection, and their sexual contacts may help to decrease the incidence of infections in neonates, NGU in male partners, PID with its subsequent involuntary infertility, and other diseases possibly caused by that organism.

We thank Dr Floyd H Taylor, for his advice concerning the statistical analysis, Janet Fraino and Angela Recio for technical help, and Diane Marchionda for help in preparing the manuscript.

This paper was presented in part at the 1983 sexually transmitted disease national conference held in Dallas, Texas, USA on 8 to 11 March 1983.

References

1. Taylor-Robinson D, Thomas BJ. The role of *Chlamydia trachomatis* in genital tract and associated diseases. *J Clin Pathol* 1980;**33**:205-33.
2. Smith TF, Weed LA, Petterson GR, Segura JW. Recovery of chlamydia and genital mycoplasma transported in sucrose phosphate buffer and urease color test medium. *Health Laboratory Science* 1977;**14**:30-4.
3. Albers AC, Sniffen JM, Freedel DN, *et al.* Selective pigment medium for *Streptococcus agalactiae*. *Am J Med Technol* 1983;**49**:807-11.
4. Facklam RR. Streptococci. In: Lennette EH, Spaulding EH, Truant JP, eds. *Manual of clinical microbiology*. 2nd ed. Washington DC: American Society for Microbiology, 96-108.
5. Shepard MC, Lunceford CD. Differential agar medium (A7) for identification of *Ureaplasma urealyticum* (human T mycoplasmas) in primary cultures of clinical material. *J Clin Microbiol* 1976;**3**:613-25.
6. Shepard MC, Lunceford CD. Urease color test medium U-9 for the detection and identification of 'T' mycoplasma in clinical material. *Applied Microbiology* 1970;**20**:539-43.
7. Evans RT, Taylor-Robinson D. Comparison of various McCoy cell treatment procedures used for detection of *Chlamydia trachomatis*. *J Clin Microbiol* 1979;**10**:198-201.
8. Burns DCM, Darougar S, Thin RN, Lothian L, Nicol CS. Isolation of *Chlamydia* from women attending a clinic for sexually transmitted disease. *British Journal of Venereal Diseases* 1975;**51**:314-8.
9. Arya OP, Mallinson H, Goddard AD. Epidemiological and clinical correlates of chlamydial infection of the cervix. *British Journal of Venereal Diseases* 1981;**57**:118-24.
10. McCormack WM, Alpert S, McComb DE, Nichols RL, Samine DZ, Zinner SH: Fifteen-month follow-up study of women infected with *Chlamydia trachomatis*. *N Engl J Med* 1979;**300**:123-5.
11. Saltz GR, Zinnemann CC JR, Brookman RR, Rauh JZ. *Chlamydia trachomatis* cervical infections in female adolescents. *J Pediatr* 1981;**98**:981-5.
12. Hammerschlag MR, Anderka M, Semine DZ, McComb D, McCormack WC. Prospective study of maternal and infantile infection with *Chlamydia trachomatis*. *Pediatrics* 1979;**64**:142-8.
13. Heggie AD, Zumicao GG, Stuart LA, Gyaves MT. *Chlamydia trachomatis* infection in mothers and infants: a prospective study. *Am J Dis Child* 1981;**135**:507-11.
14. Martin DH, Koutsky L, Eschenbach DA, *et al.* Prematurity and perinatal mortality in pregnancies complicated by maternal *Chlamydia trachomatis* infections. *JAMA* 1982;**247**:1585-8.
15. Frommell GT, Rothenberg R, Wang SP, *et al.* Chlamydial infection of mothers and their infants. *J Pediatr* 1979;**95**:28-32.
16. Harrison HR, Alexander WE, Weinstein L, Lewis M, Sim DA. Epidemiological correlation of genital infections and outcomes in pregnancy. In: Mårdh P-A, Holmes KK, Oriol DJ, Piot P, Schachter J, eds. *Chlamydial infections*. Amsterdam: Elsevier Biomedical Press, 1982:159-62.
17. Harrison HR, Boyce WT, Haffner WHJ, *et al.* The prevalence of genital *Chlamydia trachomatis* and mycoplasmal infection during pregnancy in an American Indian population. *Sex Transm Dis* 1983;**10**:184-6.
18. Thompson S, Lopez B, Wong KH, *et al.* A prospective study of chlamydia and mycoplasma infection during pregnancy: relation to pregnancy outcome and maternal morbidity. In: Mårdh P-A, Homes KK, Oriol DJ, Piot P, Schachter J, eds. *Chlamydial infections*. Amsterdam: Elsevier Biomedical Press, 1982:155-8.
19. Mårdh P-A, Helin I, Bobeck S, Laurin J, Nilsson T. Colonisation of pregnant and puerperal women and neonates with *Chlamydia trachomatis*. *British Journal of Venereal Diseases* 1980;**56**:96-100.
20. Moller BR, Ahrons S, Laurin J, Mårdh P-A. Pelvic infection after elective abortion associated with *Chlamydia trachomatis*. *Obstet Gynecol* 1982;**59**:210-3.
21. Osser S, Persson K. Postabortal pelvic infection associated with *Chlamydia trachomatis* and the influence of humoral immunity. *Am J Obstet Gynecol* 1984;**150**:699-703.
22. Qvigstad E, Skaug K, Jerve F, Fylling P, Ulstrup JC. Pelvic inflammatory disease associated with *Chlamydia trachomatis* infection after therapeutic abortion: a prospective study. *British Journal of Venereal Diseases* 1983;**59**:189-92.
23. Qvigstad E, Skaug K, Jerve F, Vik ISS, Ulstrup JC. Therapeutic abortion and *Chlamydia trachomatis* infection. *British Journal of Venereal Diseases* 1982;**58**:182-3.
24. McCormack WM, Rosner B, Lee YH. Colonisation with genital mycoplasmas in women. *Am J Epidemiol* 1973;**97**:240-5.
25. McCormack WM, Almeida PC, Bailey PE. Sexual activity and vaginal colonisation with genital mycoplasmas. *JAMA* 1972;**221**:1375-7.
26. Tam MR, Stamm WE, Handsfield HH, *et al.* Culture-independant diagnosis of *Chlamydia trachomatis* using monoclonal antibodies. *N Engl J Med* 1984;**310**:1146-50.
27. Amortegui AJ, Meyer MP. Enzyme immunoassay for detection of *Chlamydia trachomatis* from the cervix. *Obstet Gynecol* 1985;**65**:523-6.